5. SUMMARY AND CONCLUSION

5.1. Grain and flour characteristics

Thirty lentil and forty eight horse gram lines were evaluated for different grain and flour characteristics including Hunter color, composition, protein secondary structure, gel electrophoresis and minerals. Horse gram grains showed higher a* and b* and lower L* values as compared to lentil grains. Protein content of flours from lentil and horse gram lines varied from 19.3 to 27.7% and 20.29 to 26.79%, respectively. On the other hand, ash content of lentil and horse gram flours varied from 2.16 to 3.55% and 2.71 to 4.64%, respectively. Lentil flours showed higher protein and lower ash content as compared to horse gram flours. Cu, Mn, Fe, K, Mg, Na, Zn and Ca content of flours from lentil lines varied from 0.136 to 2.36, 0.178 to 0.594, 1.029 to 2.38, 69.7 to 339, 12.8 to 47.5, 14.7 to 201, 1.56 to 10.3 and 14 to 533 mg/kg, respectively and that of flours from horse gram lines ranged from 0.105 to 1.862, 0.226 to 0.959, 0.286 to 1.514, 98 to 195, 17 to 100, 18 to 177, 1.4 to 37.9 and 35 to 300 mg/kg. These minerals were present in the following order of concentration in flours K > Ca > Na > Mg > Zn > Fe > Mn > Cu.

Proportion of antiparallel β sheets, β sheets, random coils, α-helix, β-turns and β-structures ranged from 3.64 to 6.19, 24.2 to 28.2, 12.3 to 17.5, 13.5 to 18.8, 25.3 to 32.5, and 8.78 to 17.4 respectively, for lentil lines whereas it ranged from 3.96 to 5.26, 25.2 to 28.8, 12.7 to 16.17, 13.4 to 17.4, 25.2 to 28.6 and 9.76 to 13.2 respectively for horse gram lines. Proportion of β-sheets and β-turns was the highest followed by α-helix and that of antiparallel β-sheets was the lowest. SDS-PAGE analysis of total seed storage proteins of lentil showed presence of 24-25 polypeptide (PP) subunits ranging from 90-9 kDa and that of horse gram showed 15-18 PP subunits ranging between 89-10 kDa. Low molecular weight (LMW) protein subunits ranging from 20 kDa to >10 kDa were low in horse gram flours as compared to lentil flours. Major polymorphism was found among high and medium molecular weight proteins in horse gram whereas lentil lines showed variation among LMW proteins as well. Horse gram lines showed major variation in PP subunits of 22, 23, 30-35 and 40-45 kDa molecular weight. On the other hand, lentil varieties showed major polymorphism among 10, 35-37 and 55-49
kDa PP subunits. Lentil and horse gram lines which showed variation in electrophoretic banding pattern were selected for amino acid analysis. Lentil flours showed higher proportion of aspartic acid, glutamic acid, asparagine, serine, citrulline and serine and lower proportion of histidine, threonine, GABA, tyrosine and cystine as compared to horse gram.

5.2. Functional properties of albumins and globulins

Albumins and globulins were extracted from defatted flour of lentil and horse gram. Functional properties and amino acid composition of both the fractions were determined. Albumins from both the pulses showed better FC and FS as compared to globulins. WAC of albumins and globulins was 0.791 (horse gram) and 0.397 (lentil) and 1.368 (horse gram) and 1.192 (lentil), respectively. Higher WAC and OAC were observed for horse gram proteins as compared to lentil proteins. Albumins from both the pulses showed lower WAC as compared to that of globulins. Globulins from both the pulses showed higher EAI than that of albumin. On the other hand, horse gram proteins showed lower EAI as compared to respective lentil proteins. Albumins and globulins from horse gram showed zeta potential of -23.6 and -25.4 mV, respectively whereas similar fractions from lentils showed -24.2 and -25.7 mV, respectively. Lentil proteins showed higher zeta potential as compared to horse gram proteins. Albumins from both the pulses showed the highest proportion of threonine followed by GABA whereas globulins showed the highest proportion of alanine in both the pulses. In both the proteins cystine was present in the lowest proportion as compared to other amino acids. Globulins from both the pulses showed higher proportion of serine, aspartic acid, alanine, methionine, valine, lysine and proline as compared to their respective albumins. On the other hand, albumins from both the pulses showed higher glutamine, glycine, threonine, GABA and cystine content as compared to globulins.

5.3. Interaction of protein fractions with starch and polyphenols

Interaction between protein fractions and starch was evaluated by determining the affect of their interaction on starch rheology and protein digestibility. Lentil albumins and globulins showed higher IVPD as compared to respective horse gram protein fractions. Globulins from both the pulses showed higher IVPD as compared to albumins. Both
Summary and Conclusion

albumins and globulins in the presence of starch showed improvement in IVPD. $G'$ and $G''$ of starches from both the pulses increased with increase in temperature and after reaching its maximum value i.e. $G'_{Peak}$ and $G''_{Peak}$ both the moduli decreased. $G'_{Peak}$ and $G''_{Peak}$ of starches from both the pulses were higher as compared to starch-protein blends. $G'$ and $G''$ of starches from both the pulses decreased on addition of both the proteins. Incorporation of globulin in starch caused greater decrease in $G'_{Peak}$ and $G''_{Peak}$ in comparison to that observed with albumin in both the pulses. Horse gram starch showed higher $G'_{Peak}$ and $G''_{Peak}$ as compared to lentil starch. $G'_{Final}$ and $G''_{Final}$ was the highest for horse gram starch and the lowest for starch-globulin pastes of lentil. Addition of globulin for both the pulses into starch pastes reduced $G'_{Final}$ and $G''_{Final}$ to greater extent as compared to that observed with albumin. Globulin gels from lentil showed higher $G'$ and $G''$ during cooling as compared to similar gels from horse gram. Starch gels from horse gram showed greater retrogradation tendency as compared to starch gels from lentil. The retrogradation tendency of starch gels from horse gram decreased on addition of proteins; this decrease was greater in starch gels incorporated with albumin. On the other hand, starch gels from lentils incorporated with globulins showed the highest retrogradation tendency. On interacting with polyphenols disgestibility of both albumins and globulins decreased in both the pulses.

5.4. Effect of extrusion condition on extrudate characteristics

Grit of lentil and horsegram was extruded at different feed moisture and extrusion temperatures. The extrudates obtained were evaluated for different characteristics. Expansion ratio increased with decrease in FM and increase in ET. $L^*$ values of both lentil and horse gram extrudates decreased with increase in FM. Lentil extrudates had higher $L^*$ values as compared to horse gram indicating lentil extrudates were lighter in color. $L^*$ value of lentil extrudates increased progressively with increase in ET. The $a^*$ value increased progressively with increase in FM and ET for lentil extrudates. On the other hand, $a^*$ value for horse gram extrudates decreased on increasing FM from 15 to 20% but increased on further increase in FM. Increase in ET resulted in increase in $a^*$ values of horse gram extrudates. The $a^*$ value of lentil extrudate was more as compared to horse gram extrudates. Lentil extrudates showed decrease in $b^*$ value with increase in
Summary and Conclusion

FM upto 20% but increased on further increase in moisture. However, horse gram extrudates showed progressive decrease in $b^*$ value with increase in FM. The $b^*$ value of extrudates from lentils was higher as compared to those from horse gram. WSI of lentil extrudates decreased with increase in ET. WSI of lentil and horse gram extrudates decreased with increase in FM except in lentil at 125 and 150 °C. Lentil extrudates showed decrease in WAI with increase in ET. On the contrary, WAI of horse gram extrudates increased with increase in ET. WSI of lentil and horse gram extrudates decreased with increase in FM except in lentil at 125 and 150 °C. Lentil extrudates showed decrease in WAI with increase in ET. On the contrary, WAI of horse gram extrudates increased with increase in ET. IVPD increased with decrease in FM and increase in ET. The viscosity of extrudate flour measured at 50 °C from both the pulses decreased progressively with increase in FM. BV and $\lambda_{\text{max}}$ of both lentil and horse gram extrudates decreased with increase in both ET and FM. WAC of both lentil and horse gram extrudates decreased with increase in FM and ET. PS of both the pulses increased with increase in FM. Both FC and FS increased with increase in FM for lentils. Horse gram showed an increase in FC and decrease in FS as FM was raised.

5.5. Effect of germination on flour, protein and starch characteristics

Lentil and horsegram grains were germinated for 2 (48 h) and 4 (96 h) days and effects of germination on flour protein and starch characteristics were determined. An increase in protein content with the increase in germination duration was observed in both the pulses. Ash content of both the pulses decreased with the increase in germination period. Affect of germination on the intensity of major PPs from both the pulses was significant and were clearly differentiable. LMW proteins increased and HMW proteins decreased with germination period in both the pulses. The intensity of PPs of 19 and 14 kDa increased and 80, 69, 60, 58, 55-45 and 37-33 kDa decreased in lentils with increase in germination period. PPs 87, 80, 72 and 32 kDa PPs were present in comparatively lower amount in horse gram after germination. On the other hand, PPs of 42-44 kDa were observed in negligible amount in lentils after germination. PPs of 25-22 kDa, which were not originally present in horse gram, appeared upon germination whereas 44 and 40 kDa PPs completely disappeared. PPs of 19, 26 and 29 kDa from horse gram flours were not affected by germination and seemed to be resistant to proteolysis during germination.
Summary and Conclusion

FC of both the pulses increased progressively with increase in germination duration. FS of both lentil and horse gram decreased with increase in germination duration. WAC of both the pulses increased significantly after germination. EAI decreased with increase in germination duration. Proportion of β-sheets and β-turns were the highest followed by random coils and α-helix and proportion of antiparallel β-sheets was the lowest. Proportion of anti-parallel β-sheets and β-sheets increased whereas that of α-helix and β-turns decreased with progress in germination of both the pulses; however, there were exceptions to these trends. NIC17552 showed initial increase followed by a decrease in β-turns with progress in germination.

Horse gram flour showed variable trend in PV whereas lentil flour showed improvement upon 48h of germination followed by a decline after 96h of germination. BV of lentil flours increased with germination whereas horse gram flour did not show any significant change. BV for horse gram flours was significantly higher as compared to lentil flours. SBV of both the pulses decreased with increase in germination duration. Germination decreased FV of both the pulses. Horse gram lines showed higher PT as compared to lentil lines. Germination resulted in decrease in apparent amylose content. AAC of horse gram was higher as compared to lentil in starch isolated from both germinated and ungerminated grains. Granule size of starch was in the range of 53 to 10μm and 62 to 13μm, respectively for lentil and horse gram. Average granule size of horse gram starch was higher than that of lentil. Proportion of granules ranging from 25 to 75μm and above decreased with increase in germination duration for both the pulses. PV, BV, SBV, FV and PT values decreased with increase in germination duration for both the pulses. Horse gram starch showed higher PV, BV, FV, PT and lower SBV values as compared to lentil starch. The starch from ungerminated pulses was characterized to have smooth surface. Horse gram starches were elliptical to kidney bean shaped whereas lentils were round to oval shaped. After germination for 48 to 96 h, visible changes were observed on surface of starch granules. The surface of starch granules became rough and slightly corroded with germination.

The $G'$ of cooked starch gels increased with decrease in temperature from 80 to 10°C in both the pulses. This increase in $G'$ of starch gels from lentil was higher as compared to
Summary and Conclusion

that of horsegram. Increase in $G'$ further increased during holding of starch gel at 10°C for both the pulses. $G'$ of starch gels initially decreased after 48 h of germination and then increased after 96 h of germination in both the pulses. Retrogradation tendency of starch gels decreased after 24 h of germination followed by increase after 48 h of germination. Starches from both the pulses exhibited C type (mixture of A and B-type) crystalline structure. Intensity of different peaks horse gram starches was more than lentil starch indicating high crystallinity of former starch. An increase in intensity of the peak at $2\theta = 5$ was observed with increase in germination period. The intensity of peaks increased with increase in germination duration in both the pulses indicating increase in crystallinity. The onset gelatinization temperature ($T_o$), peak temperature ($T_p$) and conclusion temperature ($T_c$) of horse gram starches was higher as compared to lentil starches. Both transition temperatures and $\Delta H_{gel}$ decreased with increase in germination duration. Crystallinity of horse gram starches was higher more as compared to lentil and higher transition temperatures were also observed.

5.6. Effect of germination on phenolic composition

The germination significantly affected the quantitative and qualitative phenolic composition of both the pulses. Gallic acid and chlorogenic acid available in free form from horse gram and lentil respectively, increased with germination duration. p-Coumaric acid and ferulic acid present in acid bound fraction increased germination in both the pulses. On the other hand, p-coumaric acid and ferulic acid released on basic hydrolysis increased in lentil flours and decreased in horse gram flours. Luteolin content in basic bound fraction increased in lentil flours and decreased in horse gram flours with increase in germination period. On the other hand, acid bound fraction of lentil showed initial increase followed by decrease after 48 h of germination in luteolin content; while horse gram showed inverse trend for the same. Amount of chlorogenic acid increased in acid bound fraction and decreased in basic bound fraction with increase in germination duration for both the pulses. Catechin content increased upto 48 h and then decreased after 48 h of germination in both acid and basic bound fraction of both the pulses.
5.7. Pulse shoot and wheatgrass characteristics

Lentil and wheat were germinated and shoots were cut after 15 days. Juice was extracted from these shoots and was evaluated for different properties. LSP showed higher protein content and lower ash content as compared to WGP. Juice powder from both wheat and lentil showed higher ash and protein content than their counterpart shoot powder. WGP showed significantly higher AOA as compared to LSP. AOA of JP was more than that of SP for both wheat and lentil. WGP showed significantly higher chlorophyll content as compared to LSP. PBW343 showed the highest chlorophyll a content; whereas, HD2967 showed the highest chlorophyll b content, irrespective of the method used. Total chlorophyll content of HD2967 grown in sunlight was the highest with the maximum contribution of chlorophyll b. LJP had significantly higher Cu, Mn and Fe content as compared to WJP; however, these minerals were present in comparable amounts in WSP and LSP. LJP showed the highest Cu, Mn, Fe and Zn content followed by CJP. Cu was present in the lowest amount in both LSP and WGP as compared to other minerals. LJP had significantly lower K content as compared to WJP.

Most of the phenolic compounds were present in higher amount in base or acid bound form and their relative amount in free form was low in both LSP and WGP. WGP showed the highest amount of sinapic acid as compared to other phenolic compounds. Both LSP and WGP showed that catechin, protocatechuic acid and luteolin were absent in free form whereas reversatol and caffeic acid were present in negligible amount. WJP showed higher amount of sinapic acid, ferulic acid and p-coumaric acid and lower amount gallic acid, chlorogenic acid and vanillic acid in free form as compared to LSP. JP had higher amount of sinapic acid, ferulic acid, vanillic acid, caffeic acid, gallic acid and chlorogenic acid as compared to SP; whereas, SP showed higher amount of p-coumaric acid, reversatol and quercitin as compared to JP in free form. LGP showed comparable amounts of protocatechuic acid in both acid and base bound fraction; however, gallic acid and quercitin were present in acid bound form in LJP as compared to other sources. Acid bound fraction of WJP showed higher amount of protocatechuic acid, luteolin, sinapic acid and ferulic acid and lower amount of gallic acid, catechin and chlorogenic acid as compared to LSP. Gallic acid was present mostly in acid bound form in LJP. Base bound fraction of WGP showed higher amount of
chlorogenic acid (juice), caffeic acid, protocatechuic acid, ferulic acid and sinapic acid as compared to LSP; whereas LSP had higher amount of catechin, chlorogenic acid (shoots) and p-coumaric acid as compared to WGP. WJP had the highest proportion of uncharged amino acids whereas PJP had the highest relative proportion of hydrophobic amino acids (Table 28b). WJP had higher proportion of basic amino acids as compared to PJP. LJP showed the highest amount of acidic amino acids. WJP showed higher glutamic acid and GABA content as compared to LJP.

**Conclusion**

Lentil and horse gram are rich source of proteins, minerals and essential amino acids. β-sheets were found in the highest proportion in both the pulses. Lentils have conserved protein polypeptides when analysed electrophoretically whereas polymorphism can be observed among LMW horse gram proteins. Globulins from both the pulses showed better digestibility as compared to albumins for due to presence of lower cystine content and hence less number of disulphide bonds as compared to albumins. On incorporation of starch, both albumins and globulins showed improvement in protein digestibility. Swelling of starch from both the pulses reduced on addition of proteins on heating. Globulins reduced starch swelling to greater extent. Retrogradation tendency of starch gels also decreased in the presence of both albumins and globulins. Extrudates from both the pulses showed improved digestibility and lower ER at high FM and low ET. Majority of the PPs were degraded during extrusion cooking, however, higher FM conditions caused lower thermal degradation of proteins. Increase in FM resulted in decrease in viscosity, ER and IVPD of extrudates from both the pulses. Horsegram extrudates showed lower viscosity and ER as compared to lentil extrudates due to lower amylose content. Both the pulses extrusion cooked at low FM can be used to develop ready to eat puddings with high protein, viscosity and digestibility. Germination resulted in breakdown of HMW proteins to LMW proteins thus increasing the amount of LMW proteins having large number of polar groups. These increased polar groups on proteins further increased the WAC of the flours. Hydrolysis of large peptides into small peptides during germination improved foaming capacity of flours however did not result in stable foams. Flours germinated for 48h had higher PV and BV as compared to
Summary and Conclusion

those germinated for 96h. Germination increased proportion of β-sheets however, decreased random coils, and α-helix for both pulses. Proportion of large sized granules decreased accompanied by increase in proportion of small size granules during germination indicating breakdown of large granules by amylases with increase in germination duration. WGP showed better nutritional properties as compared to PSP. Chlorophyll content and antioxidant potential of WGP was higher than PSP. PSP however had higher protein content as compared to WGP. Most of the phenolics were present in base bound fraction and were higher in WGP as compared to PSP. Juice powder was nutritionally better that shoot powder.